

APPLICANT(S): ROSENBERG, Robert D.

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AMENDMENTS TO THE CLAIMS

Please add and amend the claims to read as follows, and cancel without prejudice or disclaimer to resubmission in a divisional or continuation application claims indicated as cancelled:

1. (Currently Amended) An A method of preparing a sulfated polysaccharide capable of binding to a protein, comprising consisting of stepwise enzymatically treating an unsulfated or incompletely sulfated polysaccharide with at least one enzyme a mixture of enzymes, said mixture of enzymes consisting of N-deacetylase-N-sulfotransferase (NDST), optionally heparitinase; optionally an epimerase, at least one O- sulfotransferase selected from the group consisting of 2-OST, 6-OST, 3-OST; optionally Δ^{4,5} glycuronidase; wherein the order of the enzymatic steps can be varied and wherein said method is enzymatic only and comprises at least one in vitro step.
2. (Currently Amended) An A method of preparing heparan sulfate, comprising consisting of stepwise enzymatically treating an unsulfated heparan synthon or incompletely-sulfated heparan sulfate precursor with at least one enzyme a mixture of enzymes, said mixture of enzymes consisting of N-deacetylase-N-sulfotransferase (NDST); optionally heparitinase; optionally an epimerase, at least one O- sulfotransferase is selected from the group consisting of 2-OST, 6-OST, 3-OST, optionally Δ^{4,5} glycuronidase, and wherein the order of the enzymatic steps can be varied and wherein said method is enzymatic only and comprises at least one in vitro step.
3. (Withdrawn) A method of preparing a sulfated polysaccharide capable of binding to a binding partner, comprising treating an unsulfated or incompletely-sulfated polysaccharide with at least one chemical reagent and at least one enzyme.
4. (Withdrawn) A method of preparing heparan sulfate, comprising treating an unsulfated heparan synthon or incompletely sulfated heparan sulfate precursor with at least one chemical reagent and at least one enzyme.
5. (Withdrawn) The method of claim 3 or 4, wherein the chemical reagent is selected from the group consisting of an *N*-deacetylation reagent, an *N*-sulfonation reagent, an epimerization reagent, and an *O*-sulfonation reagent.

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6. (Cancelled)

7. (Withdrawn) The method of claim 1, wherein said method further comprises treating the unsulfated or incompletely sulfated polysaccharide with up to four chemical reagents.

8. (Previously Presented) The method of claim 1, wherein the unsulfated or incompletely sulfated polysaccharide is treated with up to four enzymes.

9. (Withdrawn) The method of claim 2, wherein said method further comprises treating the heparan synthon or incompletely sulfated heparan sulfate precursor with up to four chemical reagents.

10. (Previously Presented) The method of claim 2, wherein the heparan synthon or incompletely sulfated heparan sulfate precursor is treated with up to four enzymes.

11. (Previously Presented) The method of claim 1, comprising:

(a) treating an unsulfated polysaccharide with an *N*-deacetylating reagent;

(b) treating the step (a) product with an *N*-sulfating reagent;

(c) treating the step (b) product with an epimerizing reagent; and

(d) treating the step (c) product with at least one *O*-sulfating reagent.

12. (Previously Presented) The method of claim 2, comprising:

(a) treating a heparan synthon with an *N*-deacetylating reagent;

(b) treating the step (a) product with an *N*-sulfating reagent;

(c) treating the step (b) product with an epimerizing reagent; and

(d) treating the step (c) product with at least one *O*-sulfating reagent.

13. (Previously Presented) The method of claim 2, wherein the heparan synthon is a non-sulfated *N*-acetyl heparosan.

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14. (Previously Presented) The method of claim 1, wherein the unsulfated polysaccharide is isolated from a cell.

15. (Previously Presented) The method of claim 1, wherein the unsulfated polysaccharide is isolated from *E. coli* bacteria.

16. (Previously Presented) The method of claim 2, wherein the heparan synthon is isolated from a cell.

17. (Previously Presented) The method of claim 2, wherein the heparan synthon is isolated from *E. coli* bacteria.

18. (Original) The method of claim 11 or 12, wherein the deacetylating reagent is selected from the group consisting of a deacetylase and *N*-deacetylase- *N*-sulfotransferase.

19. (Withdrawn) The method of claim 11 or 12, wherein the deacetylating reagent is selected from the group consisting of hydrazine and a metal hydroxide.

20. (Original) The method of claim 11 or 12, wherein the *N*-sulfating reagent is selected from the group consisting of an *N*- sulfotransferase and *N*-deacetylase- *N* sulfotransferase.

21. (Withdrawn) The method of claim 11 or 12, wherein the *N*-sulfating reagent is selected from the group consisting of trialkylamine sulfur trioxide and chlorosulfonic acid.

22. (Original) The method of claim 11 or 12, wherein the epimerizing reagent is selected from the group consisting of C5-epimerase.

23. (Original) The method of claim 11 or 12, wherein the *O*-sulfating reagent incorporates a 3-*O* sulfate group.

24. (Original) The method of claim 11 or 12, wherein the *O*-sulfating reagent incorporates a 6-*O* sulfate group.

25. (Original) The method of claim 11 or 12, wherein the *O*-sulfating reagent incorporates a 2-*O* sulfate group.

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26. (Original) The method of claim 11 or 12, wherein the at least one *O*-sulfating reagent(s) incorporates one at least one of a 2-*O* sulfate group, a 6-*O* sulfate group, and a 3-*O* sulfate group.
27. (Original) The method of claim 11 or 12, wherein the *O*-sulfating reagent is a 3-*O* sulfotransferase.
28. (Original) The method of claim 11 or 12, wherein the 3-*O* sulfotransferase is selected from the group consisting of 3-OST1, 3-OST2, 3-OST3, 3-OST3a, 3-OST4, 3-OST5 and 3-OST6.
29. (Original) The method of claim 11 or 12, wherein the *O*-sulfating reagent is a 6-*O* sulfotransferase.
30. (Original) The method of claim 11 or 12, wherein the 6-*O* sulfotransferase is selected from the group consisting of 6-OST1, 6-OST2, and 6-OST3.
31. (Original) The method of claim 11 or 12, wherein *O*-sulfating reagent is a 2-*O* sulfotransferase.
32. (Withdrawn) A method of detecting or identifying a polysaccharide binding partner, comprising:
 - (a) combining the binding partner with a polysaccharide under suitable conditions for detecting a binding partner - polysaccharide interaction,
wherein the polysaccharide is prepared according to the method of claim 1; and
 - (b) detecting or measuring said interaction.
33. (Withdrawn) A method of determining the enzymes that participate in the synthesis of a polysaccharide, comprising treating a polysaccharide one or more of at least one *N*-deacetylating reagent, *N*-sulfating reagent, epimerizing reagent, and *O*-sulfating reagent and examining the enzymatic products generated by said treatment, wherein the generation of an

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enzymatic product is indicative that the enzyme participates in the synthesis of the polysaccharide.

34. (Withdrawn) The method of claim 33, wherein the order in which enzymes participate in the synthesis of the polysaccharide is determined.

35. (Withdrawn) An enzymatically synthesized N-sulfated, 2-O sulfated, 6-O sulfated, 3-O sulfated heparan sulfate that binds to antithrombin III.

36. (Withdrawn) An enzymatically synthesized N-sulfated, 2-O sulfated, 6-O sulfated, 3-O sulfated heparan sulfate that binds to glycoprotein D of herpes simplex virus 1, wherein the 3-O sulfation was made by 3-OST3a.

36. (Withdrawn) An enzymatically synthesized N-sulfated, 6-O sulfated, 3-O sulfated heparan sulfate that binds to antithrombin III.

37. (Withdrawn) An enzymatically synthesized Polysaccharide selected from the group consisting of Polysaccharide 1, Polysaccharide 2, Polysaccharide 3, Polysaccharide 4, Polysaccharide 5, Polysaccharide 6, Polysaccharide 7, Polysaccharide 8, Polysaccharide 9, Polysaccharide 10, Hexasaccharide 11, Hexasaccharide 12, Hexasaccharide 13, Pentasaccharide 14, Pentasaccharide 15, Pentasaccharide 16, and Tetrasaccharide 17.

38. (Withdrawn) The method of claim 37, wherein the polysaccharide is a functional analogue or derivative of a pentasaccharide.

39. (Cancelled).

40. (Withdrawn) A method of purifying a polysaccharide comprising the steps of isolating the polysaccharide using mass spectrometry.

41. (Withdrawn) The method of claim 40, wherein the Polysaccharide is selected from the group consisting of Polysaccharide 1, Polysaccharide 2, Polysaccharide 3, Polysaccharide 4, Polysaccharide 5, Polysaccharide 6, Polysaccharide 7, Polysaccharide 8, Polysaccharide 9, Polysaccharide 10, Hexasaccharide 11, Hexasaccharide 12, Hexasaccharide 13, Pentasaccharide 14, Pentasaccharide 15, Pentasaccharide 16, and Tetrasaccharide 17.

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42. (Withdrawn) A method of 2-*O*-sulfating Polysaccharide 11, comprising the steps of treating Polysaccharide 11 with epimerase and 2-OST1.

43. (Previously Presented) The method of claim 1, comprising the steps of

- (a) treating a heparan synthon with an *N*-sulfating reagent;
- (b) treating the step (a) product with a heparitinase reagent;
- (c) treating the step (b) product with an epimerizing reagent and a 2-*O*-sulfating reagent;
- (d) treating the step (c) product with a 6-*O*-sulfating reagent;
- (e) treating the step (d) product with a $\Delta^{4,5}$ glycuronidase; and
- (f) treating the step (e) product with 3-*O*-sulfating reagent.

44. (Withdrawn) The method of claim 1, comprising the steps of

- (a) treating an unsulfated polysaccharide with an *N*-sulfating reagent;
- (b) treating the step (a) product with an epimerizing reagent;
- (c) treating the step (b) product with a 6-*O*-sulfating reagent; and
- (d) treating the step (c) product with nitrous acid and sodium borohydride;

wherein at least one of said reagents is an enzyme.

45. (Cancelled).

46. (Previously Presented) The method of claim 1, comprising the steps of

- (a) treating an unsulfated polysaccharide with an *N*-sulfating reagent; and
- (b) treating the step (a) product with an epimerizing reagent and a 2-*O*-sulfating reagent.

47. (Previously Presented) The method of claim 1, comprising the steps of

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(a) treating an unsulfated polysaccharide with an *N*-sulfating reagent;

(b) treating the step (a) product with an epimerizing reagent; and

(c) treating the step (b) product with a 2-*O*-sulfating reagent.

48. (Previously Presented) The method of claim 1, comprising the steps of

(a) treating an unsulfated polysaccharide with an *N*-sulfating reagent; and

(b) treating the step (a) product with an 2-*O*-sulfating reagent.

49. (Withdrawn) The method of determining the structural identification of Hexasaccharide 11, comprising the steps of

(a) treating Hexasaccharide 11 with $\Delta^{4,5}$ - β -glyuronidase; and

(b) treating the step (a) product with an α -*N*-Acetylglucosaminidase.

50. (Withdrawn) A method of producing Polysaccharide 2, the method comprising the step of treating Polysaccharide 1 with an *N*-sulfating reagent.

51. (Withdrawn) A method of producing Polysaccharide 3, the method comprising the step of treating Polysaccharide 2 with an epimerizing agent.

52. (Withdrawn) A method of producing Polysaccharide 4, the method comprising the step of treating Polysaccharide 3 with a 2-*O*-sulfating reagent.

53. (Withdrawn) A method of producing Polysaccharide 5, the method comprising the step of treating Polysaccharide 4 with a 6-*O*-sulfating reagent.

54. (Withdrawn) A method of producing Polysaccharide 6, the method comprising the step of treating Polysaccharide 5 with a 3-*O*-sulfating reagent.

55. (Withdrawn) A method of producing Polysaccharide 7, the method comprising the step of treating Polysaccharide 3 with a 6-*O*-sulfating reagent.

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56. (Withdrawn) A method of producing Polysaccharide 8, the method comprising the step of treating Polysaccharide 7 with a 3-*O*-sulfating reagent.

57. (Withdrawn) A method of producing Polysaccharide 9, the method comprising the step of treating Polysaccharide 5 with a 3-*O*-sulfating reagent.

58. (Withdrawn) A method of producing Polysaccharide 10, the method comprising the step of treating Polysaccharide 1 with an *N*-sulfating reagent.

59. (Withdrawn) A method of producing Hexasaccharide 11, the method comprising the step of treating Polysaccharide 10 with a heparitinase reagent.

60. (Withdrawn) A method of producing Hexasaccharide 12, the method comprising the step of treating Hexasaccharide 11 with an epimerase reagent and a 2-*O*-sulfating reagent.

61. (Withdrawn) A method of producing Hexasaccharide 13, the method comprising the step of treating Hexasaccharide 12 with a 6-*O*-sulfating reagent.

62. (Withdrawn) A method of producing Pentasaccharide 14, the method comprising the step of treating Hexasaccharide 13 with $\Delta^{4,5}$ - β -glyuronidase reagent.

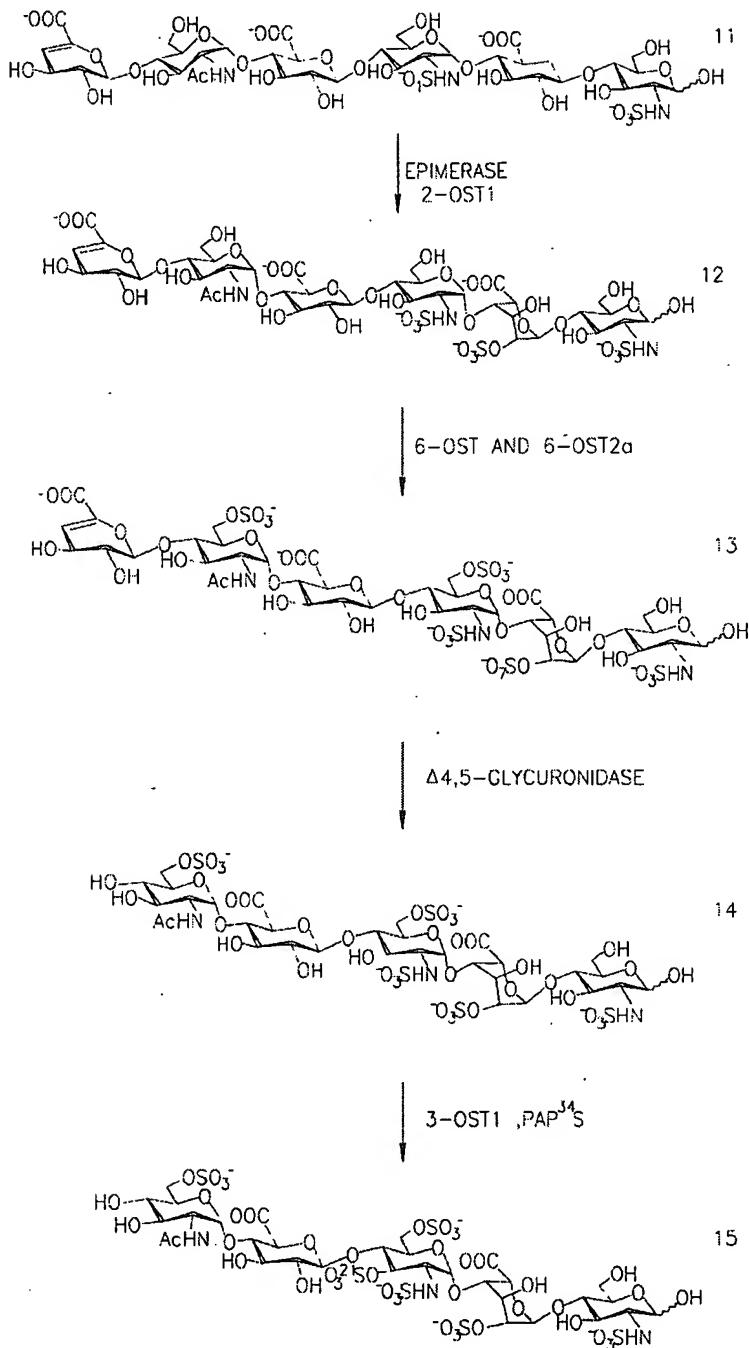
63. (Currently Amended) An *in vitro* enzymatic method of producing Pentasaccharide 15, the method comprising the step of treating Pentasaccharide 14 with a 3-*O*-sulfating reagent; ~~wherein said method is enzymatic only~~.

64. (Withdrawn) A method of producing Pentasaccharide 16, the method comprising the step of treating Pentasaccharide 11 with a $\Delta^{4,5}$ - β -glyuronidase reagent.

65. (Withdrawn) A method of producing Tetrasaccharide 17, the method comprising the step of treating Pentasaccharide 16 with a α -*N*-Acetylglucosaminidase.

66. (Currently Amended) An enzymatic method of synthesizing Pentasaccharide 15, comprising the steps of:

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wherein said method is enzymatic only and comprises at least one in vitro step.

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67. (Previously Presented) The method of claim 1, wherein the method is conducted in vitro.
68. (Previously Presented) The method of claim 2, wherein the method is conducted in vitro.
69. (Currently Amended) The method of claim 11, wherein said the method treating-is conducted in vitro.
70. (Currently Amended) The method of claim 12, wherein said the method treating is conducted in vitro.
71. (Previously Presented) The method of claim 66, wherein the method is conducted in vitro.